

**AMSTER  
ROTHSTEIN  
& EBENSTEIN LLP**  
*Intellectual Property Law*

**F A C S I M I L E**

90 Park Avenue  
New York NY 10016  
Main 212 336 8000  
Fax 212 336 8001  
Web www.arelaw.com

March 3, 2005

To: Examiner Alexander S. Noguerola  
U.S. Patent Office

Fax: 571 273 1343

Voice:

From: Alan Miller, Esq.

Re: (C/M) U.S. Patent Application No. 09/889,243  
Title: BIOSENSOR, THIN FILM ELECTRODE FORMING  
METHOD, QUANTIFICATION APPARATUS, AND  
QUANTIFICATION METHOD  
Our Ref: 55220/785

Prepd. by: Rosa Return to: Rosa No. of pages including cover: 66

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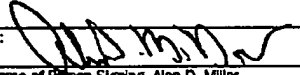
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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants : Shoji Miyazaki et al.  
Appl. No. : 09/889,243  
Filed : October 1, 2001  
For : BIOSENSOR, METHOD OF FORMING THIN-FILM  
ELECTRODE, AND METHOD AND APPARATUS FOR  
QUANTITATIVE DETERMINATION  
Examiner : Alexander S. Noguerola  
Group Art Unit : 1753

**COMMUNICATION TO SUBMIT CORRECTED SPECIFICATION PAGES**

Commissioner for Patents  
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Alexandria, VA 22313-1450

CERTIFICATE OF TRANSMISSION  
I hereby certify that this correspondence is being facsimile  
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on March 3, 2005  
Signature:   
Printed Name of Person Signing Alan D. Miller

Sir:

This Communication is submitted further to a March 2, 2005 telephone conversation between Examiner Alexander Noguerola and the undersigned attorney. The Examiner indicated that a response filed today by facsimile would be timely filed. Accordingly, this Communication is being timely filed.

303019.1

Applicants: Shoji Miyazaki et al.  
Appl. No.: 09/889,243  
Filed: October 1, 2001  
page 2

**Amendments to the Specification:**

Pursuant to MPEP §1302.02, please replace pages 8-30, 33, 41, 49, 57, 66, 75, 81 and 85 of the specification with substitute pages 8-30, 33, 41, 49, 57, 66, 75, 81 and 85 attached hereto.

Applicants also attach hereto a marked-up version of pages 8-30, 33, 41, 49, 57, 66, 75, 81 and 85 showing the changes made.

Applicants maintain that the substitute pages and amendments to the specification do not raise an issue of new matter.

Applicants note that each substitute page attached hereto begins and ends at the same point in the text as the corresponding original page of the application.

303019.1

Applicants: Shoji Miyazaki et al.  
Appl. No.: 09/889,243  
Filed: October 1, 2001  
page 3

CONCLUSION

No fee is deemed necessary in connection with the submission of this Communication. However, if any fee is required in connection with this Communication or to maintain the pendency of the subject application, authorization is hereby given to withdraw the amount of any such fee from Deposit Account No. 01-1785.

Respectfully submitted,

AMSTER, ROTHSTEIN & EBENSTEIN LLP  
Attorneys for Applicants  
90 Park Avenue  
New York, New York 10016  
(212) 336-8000

Dated: March 3, 2005  
New York, New York

By   
Alan D. Miller, Reg. No. 42,889

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the performance of the biosensor Z is deteriorated.

Further, it is considerably troublesome to insert the correction chip for every measurement, and when it is forgotten to insert the correction chip, a correction chip for example for measuring lactic acid value is inserted by mistake, or a correction chip which is for measuring blood sugar level but has different output characteristics is inserted, there occurs an error in a measured result.

The present invention is made to solve the above-mentioned problems, and has for its object to provide a biosensor which can be formed by a simple manufacturing method and has a high measuring accuracy, a biosensor in which a reagent layer is disposed uniformly on electrodes regardless of a reagent liquid composition, resulting in an uniform performance, a biosensor which enables a measuring device to discriminate correction data for each production lot only by being inserted therein without a correction chip being inserted, a thin film electrode forming method for these biosensors, as well as a method and an apparatus for quantifying using the biosensors.

#### DISCLOSURE OF THE INVENTION

According to the present invention, there is provided a biosensor for quantifying a substrate included in a sample liquid comprising: a first insulating support and a second insulating support; an electrode part comprising at

least a working electrode and a counter electrode; a specimen supply path for introducing the sample liquid to the electrode part; and a reagent layer employed for quantifying the substrate included in the sample liquid, and the electrode part, the specimen supply path, and the reagent layer exist between the first insulating support and the second insulating support, the specimen supply path is provided on the electrode part, and the reagent layer is provided on the electrode part in the specimen supply path, respectively, and the electrode part is dividedly formed by first slits provided on an electrical conductive layer which is formed on the whole or part of an internal surface of one or both of the first insulating support and the second insulating support.

Since a biosensor is constructed as described above, an electrode part can be defined easily and with a high accuracy, and variations in response of each biosensor can be reduced, resulting in a favorable response. Further, the electrode part is formed in a monolayer of electrical conductive layer, whereby troubles can be reduced and an electrode part with a smooth surface can be formed by a simple method. Since the structure of the electrode part is quite simple, it is possible to easily form biosensors having the same performance.

According to an embodiment of the present invention, in the biosensor, the electrode part further comprises a detecting electrode.

Since the biosensor is constructed as described above, it is possible to make the biosensor have a better accuracy.

According to an embodiment of the present invention, in the biosensor, the counter electrode is provided on the whole or part of the internal surface of the second insulating support, the working electrode and the detecting electrode are provided on the whole or part of the internal surface of the first insulating support, and the working electrode and the detecting electrode which are provided on the internal surface of the first insulating support are dividedly formed by the first slits provided on the electrical conductive layer.

Since the biosensor is constructed as described above, it is possible to downscale a specimen supply path, whereby a measurement can be done with a slight amount of specimen.

According to an embodiment of the present invention, in the biosensor, the electrode part is provided on the whole or part of the internal surface of only the first insulating support, and the electrode part provided on the internal surface of the first insulating support is dividedly formed by the first slits provided on the electrical conductive layer.

Since the biosensor is constructed as described above, all of the electrodes are provided on the same surface, and thus the electrodes are formed only on one surface, resulting in an

easier manufacture, whereby the manufacturing costs of the biosensor can be reduced.

According to an embodiment of the present invention, in the biosensor, an area of the counter electrode is equal to or larger than that of the working electrode.

Since the biosensor is constructed as described above, an electron transfer reaction between the counter electrode and the working electrode is prevented to be rate-determined, thereby promoting the reaction smoothly.

According to an embodiment of the present invention, in the biosensor, a total of an area of the counter electrode and an area of the detecting electrode is equal to or larger than that of the working electrode.

Since the biosensor is constructed as described above, electron transfer reactions between the counter electrode as well as the detecting electrode and the working electrode are prevented to be rate-determined, thereby promoting the reactions smoothly.

According to an embodiment of the present invention, in the biosensor, the area of the detecting electrode in the specimen supply path of the biosensor is equal to the area of the counter electrode.

Since the biosensor is constructed as described above,

electron transfer reactions between the counter electrode as well as the detecting electrode and the working electrode are more reliably prevented to be rate-determined, thereby promoting the reactions smoothly.

According to an embodiment of the present invention, in the biosensor, a spacer is provided which has a cutout part for forming the specimen supply path and is placed on the electrode part, and the second insulating support is placed on the spacer.

Since the biosensor is constructed as described above, the position where the specimen supply path is provided is fixed, and the second insulating support is placed thereon, thereby preventing the specimen introduced to the specimen supply path from leaking from the specimen supply path.

According to an embodiment of the present invention, in the biosensor, the spacer and the second insulating support is integral.

Since the biosensor is constructed as described above, the spacer and the second insulating support is integral, thereby to enable a cost reduction and a simple manufacture.

According to an embodiment of the present invention, in the biosensor, an air hole leading to the specimen supply path is formed.

Since the biosensor is constructed as described above, excessive air is discharged from the air hole when the specimen

is introduced to the specimen supply path, thereby reliably introducing the specimen to the specimen supply path due to the capillary phenomenon.

According to an embodiment of the present invention, in the biosensor, the reagent layer is formed by dripping a reagent, and second slits are provided around a position where the reagent is dripped.

Since the biosensor is constructed as described above, when the reagent is dripped on the electrodes for the reagent layer formation, thereby forming the reagent layer, the reagent spreads uniformly forming the reagent layer of a prescribed area at the prescribed position, whereby the reagent layer free from variations in the position and area can be formed, resulting in a correct measurement free from the variations.

According to an embodiment of the present invention, in the biosensor, the second slits are arc shaped.

Since the biosensor is constructed as described above, the spread of the reagent is defined by the slits which have the same shapes as that of the reagent spread, thereby defining the area and the position of the reagent layer more correctly.

According to an embodiment of the present invention, in the biosensor, third slits are provided for dividing the electrical conductive layer to define an area of the electrode part.

Since the biosensor is constructed as described above, when the support is initially cut at the manufacturing process of the biosensor, the area of each electrode is previously defined by the third slits, whereby the area of each electrode does not change due to the cut position of the support, thereby preventing variations in the accuracy.

According to an embodiment of the present invention, in the biosensor, shapes of the first insulating support and the second insulating support are approximately rectangular, and one third slit or two or more third slits are provided in parallel with one side of the approximate rectangle shape.

Since the biosensor is constructed as described above, the area of each electrode can be defined easily by the third slits, and the area of each electrode does not change due to deviations of the cut position when the support is cut, resulting in no variation in the accuracy.

According to an embodiment of the present invention, the biosensor has information of correction data generated for each production lot of the biosensor, which correspond to characteristics concerning output of an electrical change resulting from a reaction between the reagent liquid and the reagent layer and can be discriminated by a measuring device employing the biosensor.

Since the biosensor is constructed as described above, the

measuring device can discriminate which the correction data is required, only by inserting the biosensor into the measuring device, and there is no need for a user to input the information about the correction data employing a correction chip or the like, thereby removing troubles and preventing operational errors to obtain a correct result.

According to an embodiment of the present invention, in the biosensor, one or plural fourth slits dividing the electrode part are provided, and the measuring device can discriminate the information of the correction data according to positions of the fourth slits.

Since the biosensor is constructed as described above, the measuring device can discriminate the information of the correction data by the positions of the fourth slits, the correction data can be indicated correspondingly to plural production lots, the measuring device can easily discriminate which correction data is required, by inserting the biosensor into the measuring device, whereby there is no operational trouble, resulting in preventing operational errors to obtain a correct result.

According to an embodiment of the present invention, in the biosensor, at least one or all of the first slits, the second slits, the third slits, and the fourth slits are formed by processing the electrical conductive layer by a laser.

Since the biosensor is constructed as described above, a high-accuracy processing is possible, the area of each electrode can be defined with a high accuracy, and further the clearance between the respective electrodes can be narrowed, resulting in a small-size biosensor.

According to an embodiment of the present invention, in the biosensor, a slit width of respective one of the first slits, the second slits, the third slits, and the fourth slits is 0.005 mm to 0.3 mm.

Since the biosensor is constructed as described above, the clearance between the respective electrodes can be narrowed, resulting in a small-size biosensor.

According to an embodiment of the present invention, in the biosensor, a slit depth of respective one of the first slits, the second slits, the third slits, and the fourth slits is equal to or larger than the thickness of the electrical conductive layer.

Since the biosensor is constructed as described above, there can be obtained a biosensor in which the respective electrodes are surely separated.

According to an embodiment of the present invention, in the biosensor, the reagent layer includes an enzyme.

Since the biosensor is constructed as described above, there can be obtained an enzyme biosensor suitable for an

inspection which employs the enzyme.

According to an embodiment of the present invention, in the biosensor, the reagent layer includes an electron transfer agent.

Since the biosensor is constructed as described above, there can be obtained a biosensor suitable for an inspection utilizing a reaction of the electron transfer agent.

According to an embodiment of the present invention, in the biosensor, the reagent layer includes a hydrophilic polymer.

Since the biosensor is constructed as described above, there can be obtained a high-accuracy biosensor which can easily form the reagent layer.

According to an embodiment of the present invention, in the biosensor, the insulating support is made of a resin material.

Since the biosensor is constructed as described above, it is possible to manufacture a lower-cost biosensor.

According to the present invention, there is provided a thin film electrode forming method for forming a thin film electrode on a surface of an insulating support including: a roughened surface forming step of roughening the surface of the insulating support by colliding an excited gas against the surface of the insulating support in a vacuum atmosphere; and an electrical conductive layer forming step of

forming the electrical conductive layer as a thin film electrode which is composed of a conductive substance on the roughened surface of the insulating support.

Since the thin film electrode is formed as described above, a preprocessing such as a surface polishing processing is not required, whereby it is possible to form the thin film electrode by a simpler method and to form the thin film electrode with high adhesion between the support and the electrode layer.

According to an embodiment of the present invention, in the thin film electrode forming method, the roughened surface forming step comprises: a support placing step of placing the insulating support in a vacuum chamber; an evacuation step of evacuating the vacuum chamber; a gas filling step of filling up the vacuum chamber with a gas; and a colliding step of exciting the gas to be ionized and colliding the same against the insulating support.

Since the thin film electrode is formed as described above, it is possible to form the support surface suitable for forming the thin film electrode more effectively and reliably, thereby forming the thin film electrode more effectively.

According to an embodiment of the present invention, in the thin film electrode forming method, a degree of the vacuum in the evacuation step is within a range of  $1 \times 10^{-1}$  to  $3 \times 10^{-3}$  pascals.

Since the thin film electrode is formed as described above, it is possible to form the support surface suitable for forming the thin film electrode more reliably, thereby forming the thin film electrode more effectively.

According to an embodiment of the present invention, in the thin film electrode forming method, the gas is an inert gas.

Since the thin film electrode is formed as described above, the support surface can be made in a state suitable for forming the thin film electrode without denaturing the support surface.

According to an embodiment of the present invention, in the thin film electrode forming method, the inert gas is either a rare gas of argon, neon, helium, krypton, and xenon, or nitrogen.

Since the thin film electrode is formed as described above, there can be formed the thin film electrode more reliably without denaturing the support surface.

According to an embodiment of the present invention, in the thin film electrode forming method, the electrical conductive layer forming step comprises: a second support placing step of placing an insulating support having an already roughened surface, which has been subjected to the roughened surface forming step, in a second vacuum chamber; a second evacuation step of evacuating the second vacuum chamber; a second gas filling step of filling

up the second vacuum chamber with a second gas; and a step of exciting the second gas to be ionized and colliding the same against a conductive substance to beat out atoms of the conductive substances, to form a film on the insulating support having the already roughened surface.

Since the thin film electrode is formed as described above, a preprocessing such as a surface polishing processing is not required and the thin film electrode with higher adhesion to the support can be obtained.

According to an embodiment of the present invention, in the thin film electrode forming method, the electrical conductive layer forming step comprises: a second support placing step of placing an insulating support having an already roughened surface, which has been subjected to the roughened surface forming step, in a second vacuum chamber; a second evacuation step of evacuating the second vacuum chamber; and a step of heating and evaporating a conductive substance to deposit steams as a film on the insulating support having the already roughened surface.

Since the thin film electrode is formed as described above, a preprocessing such as a surface polishing processing is not required and the thin film electrode with higher adhesion to the support can be obtained.

According to an embodiment of the present invention, in the thin film electrode forming method,

a degree of the vacuum in the second evacuation step is within a range of  $1 \times 10^{-1}$  to  $3 \times 10^{-3}$  pascals.

Since the thin film electrode is formed as described above, there can be more reliably formed the thin film electrode with remarkably high adhesion to the support.

According to an embodiment of the present invention, in the thin film electrode forming method, the second gas is an inert gas.

Since the thin film electrode is formed as described above, there can be formed the thin film electrode with high adhesion to the support without denaturing the support surface and the thin film electrode itself.

According to an embodiment of the present invention, in the thin film electrode forming method, the inert gas is either a rare gas of argon, neon, helium, krypton and xenon, or nitrogen.

Since the thin film electrode is formed as described above, there can be more reliably formed the thin film electrode with high adhesion to the support without denaturing the support surface and the thin film electrode itself.

According to an embodiment of the present invention, in the thin film electrode forming method, the vacuum chamber and the second vacuum chamber is the same chamber.

Since the thin film electrode is formed as described above,

a facility for forming the thin film electrode can be simplified and thus the manufacturing cost of the thin film electrode can be reduced.

According to an embodiment of the present invention, in the thin film electrode forming method, the conductive substance is a noble metal or carbon.

Since the thin film electrode is formed as described above, the thin film electrode is composed of not a composite material but a single substance material, thereby enabling a mass manufacture of stable electrodes, which is not influenced by the manufacturing conditions and which has a less difference in material lots.

According to an embodiment of the present invention, in the thin film electrode forming method, a thickness of a formed thin film electrode is within a range of 3 nm to 100 nm.

Since the thin film electrode is formed as described above, the thickness of the electrode can be thinned as much as possible, thereby to enhance a production tact as well as reduce a manufacturing cost due to a reduction of the material cost.

According to an embodiment of the present invention, in the biosensor, the electrical conductive layer is formed by the thin film electrode forming method.

Since the biosensor is formed as described above, the thin film electrode reflects unevenness on the support surface which is processed into a roughened surface, so that the wettability and adhesiveness between the electrode and the reagent is enhanced, resulting in a high performance biosensor.

According to an embodiment of the present invention, there is provided a quantification method for quantifying, by employing the biosensor, a substrate included in a sample liquid supplied to the biosensor comprising: a first application step of applying a voltage between the detecting electrode and the counter electrode or the working electrode; a reagent supplying step of supplying the sample liquid to the reagent layer; a first change detecting step of detecting an electrical change occurring between the detecting electrode and the counter electrode or the working electrode by the supply of the sample liquid to the reagent layer; a second application step of applying a voltage between the working electrode and the counter electrode as well as the detecting electrode after the electrical change is detected in the first change step; and a current measuring step of measuring a current generated between the working electrode and the counter electrode as well as the detecting electrode, to which the voltage is applied in the second application step.

Since the quantification is performed as described above, the quantification operation is started when the electrical

change occurs between the detecting electrode and the working electrode or the counter electrode of the biosensor, thereby preventing measuring errors due to the shortage of the specimen amount supplied to the reagent layer, resulting in a higher accuracy measurement. Further, when the measurable amount of specimen is supplied to the reagent layer, the measurement is performed by using the detecting electrode also as the counter electrode, thereby making the area of the electrode part smaller, and thus a quantitative analysis based on a slight amount of specimen can be performed correctly.

According to an embodiment of the present invention, there is provided a quantification method for quantifying, by employing the biosensor, a substrate included in a sample liquid supplied to the biosensor comprising: a third application step of applying a voltage between the detecting electrode and the counter electrode or the working electrode as well as between the working electrode and the counter electrode; a reagent supplying step of supplying the sample liquid to the reagent layer; a first change detecting step of detecting an electrical change occurring between the detecting electrode and the counter electrode or the working electrode by the supply of the sample liquid to the reagent layer; a second change detecting step of detecting an electrical change occurring between the working electrode and the counter electrode by the supply of the sample

liquid to the reagent layer; a second application step of applying a voltage between the working electrode and the counter electrode as well as the detecting electrode after the electrical changes are detected in the first change detecting step and the second change detecting step; and a current measuring step of measuring a current generated between the working electrode and the counter electrode as well as the detecting electrode, to which the voltage is applied in the second application step.

Since the quantification is performed as described above, the quantification operation is started when the electrical change occurs between the detecting electrode and the working electrode or the counter electrode of the biosensor, thereby preventing measuring errors due to the shortage of the specimen amount supplied to the reagent layer, resulting in a higher accuracy measurement. Further, when the measurable amount of specimen is supplied to the reagent layer, the measurement is performed by using the detecting electrode also as the counter electrode, thereby making the area of the electrode part smaller, and thus quantitative analysis based on a slight amount of specimen can be performed correctly.

According to an embodiment of the present invention, in the quantification method, the second change detecting step is followed by a no-change informing step of informing a user that no change occurs when it is detected

that no electrical change occurs between the detecting electrode and the counter electrode or the working electrode for a prescribed period of time.

Since the quantification is performed as described above, it is possible to inform a user that there is a shortage of the specimen amount supplied to the reagent layer of the biosensor, resulting in the quantification method with enhanced convenience and safety.

According to an embodiment of the present invention, there is provided a quantification apparatus, to which the biosensor is detachably connected and which quantifies a substrate included in a sample liquid supplied to the biosensor comprising: a first current/voltage conversion circuit for converting a current from the working electrode included in the biosensor into a voltage; a first A/D conversion circuit for digitally converting the voltage from the current/voltage conversion circuit; a first switch provided between the counter electrode included in the biosensor and the ground; and a control part for controlling the first A/D conversion circuit and the first switch, and the control part applies a voltage between the detecting electrode and the working electrode in a state where the first switch is insulated from the counter electrode, detects an electrical change between the detecting electrode and the working electrode occurring by the sample liquid which is supplied to

the reagent layer on the specimen supply path, thereafter applies a voltage between the working electrode and the counter electrode as well as the detecting electrode in a state where the first switch is connected to the counter electrode, and measures a response current generated by applying the voltage.

Since the quantification apparatus is constructed as described above, measuring errors due to the shortage of the specimen amount supplied to the reagent layer of the specimen supply path are prevented, resulting in a higher accuracy measurement. Further, the detecting electrode of the biosensor is used also as the counter electrode at the measuring, so that the specimen supply path can be downscaled, thereby to perform a quantitative analysis of a slight amount of specimen correctly.

According to an embodiment of the present invention, there is provided a quantification apparatus, to which the biosensor is detachably connected and which quantifies a substrate included in a sample liquid supplied to the biosensor comprising: a first current/voltage conversion circuit for converting a current from the working electrode included in the biosensor into a voltage; a second current/voltage conversion circuit for converting a current from the detecting electrode included in the biosensor into a voltage; a first A/D conversion circuit for digitally converting the voltage from the first current/voltage

conversion circuit; a second A/D conversion circuit for digitally converting the voltage from the second current/voltage conversion circuit; a first selector switch for switching the connection of the detecting electrode of the biosensor to the first current/voltage conversion circuit or the ground; and a control part for controlling the first A/D conversion circuit, the second A/D conversion circuit, and the first selector switch, and the control part applies a voltage between the detecting electrode and the counter electrode as well as between the working electrode and the counter electrode in a state where the first selector switch is connected to the first current/voltage conversion circuit, detects an electrical change between the detecting electrode and the working electrode as well as an electrical change between the working electrode and the counter electrode, respectively, occurring by the sample liquid which is supplied to the reagent layer provided on the specimen supply path, thereafter connects the first selector switch to the ground, applies a voltage between the working electrode and the counter electrode as well as the detecting electrode, and measures a response current generated by applying the voltage.

Since the quantification apparatus is constructed as described above, measuring errors due to the shortage of the specimen amount supplied to the reagent layer of the specimen supply path are prevented, resulting in a higher accuracy

measurement. Further, the detecting electrode of the biosensor is used also as the counter electrode at the measuring, so that the specimen supply path can be downscaled, thereby to perform a quantitative analysis of a slight amount of specimen correctly.

According to an embodiment of the present invention, the quantification apparatus comprises: a second selector switch for switching the connection of the working electrode of the biosensor to the second current/voltage conversion circuit or the ground, and the control part applies a voltage between the detecting electrode and the counter electrode as well as between the working electrode and the counter electrode in a state where the first selector switch is connected to the first current/voltage conversion circuit and the second selector switch is connected to the second current/voltage conversion circuit, respectively, connects the second selector switch to the ground when detecting an electrical change between the working electrode and the counter electrode, occurring by the sample liquid which is supplied to the reagent layer provided on the specimen supply path, and when thereafter detecting an electrical change between the detecting electrode and the working electrode, in a state where the second selector switch is connected to the second current/voltage conversion circuit and the first selector switch is connected to the ground, applies a voltage

between the working electrode and the counter electrode as well as the detecting electrode, and measures a response current generated by applying the voltage.

Since the quantification apparatus is constructed as described above, measuring errors due to the shortage of the specimen amount supplied to the reagent layer of the specimen supply path are prevented, resulting in a higher accuracy measurement. Further, the detecting electrode of the biosensor is used also as the counter electrode at the measuring, so that the specimen supply path can be downscaled, thereby to perform a quantitative analysis of a slight amount of specimen correctly.

According to an embodiment of the present invention, the quantification apparatus comprising an informing means for informing a user that no change occurs, when the sample liquid is supplied to the reagent layer of the specimen supply path, and the control part detects that an electrical change occurs between the working electrode and the counter electrode but no electrical change occurs between the detecting electrode and the working electrode or the counter electrode.

Since the quantification apparatus is constructed as described above, it is possible to inform a user of the shortage of the specimen amount supplied to the reagent layer of the specimen supply path of the biosensor, resulting in the

blood glucose concentration of 40-600 mg/dl are compared.

Figures 21(a)-(b) are exploded perspective views of a conventional biosensor.

Figure 22 is a diagram illustrating a state where a biosensor is inserted in a measuring device.

Figure 23 is a top view illustrating a state where slits are formed in the electrical conductive layer which is provided on a sensor wafer according to the third embodiment.

Figures 24(a)-(c) are top views illustrating states of electrodes of a biosensor in a manufacturing method according to the third embodiment.

Figure 25 is a diagram illustrating the concept of a cross-sectional structure of a conventional biosensor.

#### BEST MODE TO EXECUTE THE INVENTION

Hereinafter, embodiments of the present invention will be described with reference to the figures. The embodiments which are described here are merely examples, and the present invention is not necessarily restricted thereto.

##### (Embodiment 1)

A biosensor A as defined in the present invention will be described as a first embodiment with reference to the figures.

Figures 1(a) to 1(c) are exploded perspective views of the biosensor A according to the first embodiment of the present

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conductive material such as noble metal for example gold or palladium and carbon as the material, it take no trouble of successively printing and laminating a silver paste, a carbon paste and the like on the support 1 as in the prior art, whereby it is possible to form the electrode part with a smooth surface by a simple method. Further, since the slits 4a and 4b are formed on the conductive layer 2 which is provided on the support 1 by the laser, it is possible to define the area of each electrode with a higher accuracy. The clearance between the respective electrodes can be considerably reduced to downsize the specimen supply path, thereby enabling the measurement based on a trace quantity of specimen while this could not be measured conventionally. Further, since the structures of the electrodes are very simple, a biosensor having the same performance can be easily formed.

(Embodiment 2)

A biosensor B according to the present invention will be described as a second embodiment.

Figures 3(a)-(c) are perspective views illustrating the biosensor B in the order of the manufacturing process, and figure 4 is a diagram illustrating a specimen supply path of the biosensor B.

First, the structure of the biosensor B will be described.

Numeral 21 denotes an insulating support which is composed of polyethylene terephthalate or the like. Numeral 22 denotes an electrical conductive layer which is formed on the whole

individual biosensor.

However, the so-formed biosensor X has a problem in that when the plural biosensors are to be cut into individual biosensors, there are some cases where the cutting cannot be performed on the cutting plane lines, resulting in deviations from the cutting plane lines 3110. This will be described in more detail. Figure 24(a) is a diagram illustrating states of the electrodes in a case where the cutting is correctly performed. Figure 24(b) is a diagram illustrating states of the electrodes when the cutting position is deviated toward left from the cutting plane line 3110. Figure 24(c) is a diagram illustrating states of the electrodes when the cutting position is deviated toward right from the cutting plane line 3110. Since the areas of the working electrode 3105 and the counter electrode 3106 are decided by the cutting position of the individual wafer Q, changes in the areas of the working electrode 3105 and the counter electrode 3106 occur when the cutting position is deviated from the cutting plane line 3110 as shown in the figures, resulting in variations in resistance values of the respective electrodes. Therefore, values of currents flowing the electrodes change, whereby the accuracy of the biosensor X get worse.

Here, a biosensor C according to the present invention, which has for its object to solve this problem will be described as a third embodiment.

(Embodiment 4)

A biosensor D according to the present invention will be described as a fourth embodiment.

Figure 9(a)-(c) are perspective views illustrating the biosensor D in the order of a manufacturing process. Figures 10(a)-(h) are top views exemplifying the formation of fourth slits of the biosensor D. Figure 22 is a diagram illustrating a state where the biosensor D is inserted into a measuring device.

First, components of the biosensor D will be described.

Numeral 61 denotes an insulating support composed of polyethylene terephthalate or the like. Numeral 62 denotes an electrical conductive layer which is formed on the whole surface of the support 61 and is composed of an electrical conductive material such as a noble metal, for example gold or palladium, and carbon. Numerals 63a, 63b, 63c and 63d denote first slits provided in the electrical conductive layer 62. Numerals 65, 66, and 67 denote electrodes which are formed by dividing the electrical conductive layer 62 by the first slits 63a, 63b, 63c and 63d, i.e., a working electrode, a counter electrode, and a detecting electrode as an electrode for confirming whether the specimen is surely drawn into a specimen supply path, respectively. Numerals 64a, 64b, and 64c denote fourth slits which divide the counter electrode 66, the detecting electrode 67, and the working electrode 65, respectively. Numeral 68 denotes a spacer which covers the

preferable that each slit provided on the electrical conductive layer is processed by the laser, the width of each slit is 0.005 mm - 0.3 mm, and the depth of each slit is equal to or larger than the thickness of the electrical conductive layer.

Further, it is preferred that the reagent layer provided in any of the biosensors A, B, C, and D should include enzyme, an electron transfer agent, or a hydrophilic polymer.

In addition, it is preferable that the insulating support employed in any of the biosensors A, B, C, and D is made of a resin material.

(Embodiment 5)

A thin film electrode forming method of the present invention will be described as a fifth embodiment with reference to the figures. When the thin film electrode method described in the fifth embodiment is applied when the electrode parts of any of the biosensors A, B, C, and D according to the above-described first to fourth embodiments are formed, a biosensor of the present invention can be obtained.

Figure 11 is a schematic diagram showing a state of a biosensor, where a thin film electrode is formed by implementing the thin film electrode forming method according to this embodiment and a reaction reagent layer are laid out

substrate and a quantification apparatus for quantifying a substrate, which employ any of the biosensors A, B, C, and D, for which the electrical conductive layers are formed by employing the above-described thin film electrode forming method according to the fifth embodiment will be described. while the biosensor A as described in the first embodiment is used as a biosensor employed in a following description, the biosensor to be used is not restricted thereto.

Figure 13 is a diagram illustrating structures of the biosensor and the quantification apparatus which is employed in the quantification method employing the biosensor. In the figure, the same reference numerals as those shown in figure 1 denote the same or corresponding parts.

It is a system in which the biosensor A is used in a state where it is connected to a quantification apparatus M1, and the quantification apparatus M1 measures the amount of an included substrate from a specimen supplied to the biosensor A.

In the quantification apparatus M1, numerals 115a, 115b, and 115c denote connectors connected to a working electrode 5, a detecting electrode 7, a counter electrode 6 of the biosensor A, respectively, numeral 116a denotes a switch provided between the connector 115c and the ground (which means a constant potential electrodeposition and can be not always "0". The same goes for in the present specification.), numeral 118a

electrode 7 is at least equivalent to the area of the working electrode 5, the electron transfer reaction between the electrodes is prevented from being rate-determined, thereby to promote the reaction smoothly. At the same time, the capacity of the specimen supply path can be downsized, whereby the quantitative analysis based on a slight amount of specimen, which was conventionally impossible, can be performed properly. Further, when the area of the detecting electrode 7 and that of the counter electrode 6 are equivalent, the electron transfer reaction between the electrodes is performed uniformly, thereby obtaining a more satisfactory response.

(Embodiment 7)

Hereinafter, a quantification method for quantifying a substrate and a quantification apparatus for quantifying a substrate, which employ any of the biosensors A to D whose electrical conductive layers are formed by employing the thin film electrode forming method described in the fifth embodiment but which are different from those of the above-described sixth embodiment will be described. A biosensor which is employed in a following description is supposed to be the biosensor A described in the first embodiment.

Figure 16 is a diagram illustrating structures of the biosensor A and a quantification apparatus employed in the

the counter electrode 6 and the working electrode 5 by the supply of the specimen to the specimen supply path but no current is thereafter generated between the counter electrode 6 and the detecting electrode 7 for the prescribed period of time, the CPU 120 judges that there is a shortage of the specimen amount, and this is displayed on the LCD 121. Even when the specimen is supplemented to the specimen supply path after the LCD 121 once displays that there is a shortage of the specimen supply, the CPU 120 does not start the quantification operation.

As described above, according to the quantification method employing the biosensor in the seventh embodiment of the present invention, when the specimen is drawn into the specimen supply path of the biosensor A, and electrical changes occur between the counter electrode 6 and the working electrode 5 while no electrical change occurs between the counter electrode 6 and the detecting electrode 7, the quantification apparatus M3 displays on the LCD 121 that there is a shortage of the specimen supply and informs a user of the fact, thereby enhancing the convenience and safety at the measuring.

(Embodiment 8)

Hereinafter, a quantification method for quantifying a substrate and a quantification apparatus for quantifying a substrate, which employ any of the biosensors A to D whose electrical conductive

the performance of the biosensor 2 is deteriorated.

Further, it is considerably troublesome to insert the correction chip for every measurement, and when it is forgotten to insert the correction chip, a correction chip for example for measuring lactic acid value is inserted by mistake, or a correction chip which is for measuring blood sugar level but has different output characteristics is inserted, there occurs an error in a measured result.

The present invention is made to solve the above-mentioned problems, and has for its object to provide a biosensor which can be formed by a simple manufacturing method and has a high measuring accuracy, a biosensor in which a reagent layer is disposed uniformly on electrodes regardless of a reagent liquid composition, resulting in an uniform performance, a biosensor which enables a measuring device to discriminate correction data for each production lot only by being inserted therein without a correction chip being inserted, a thin film electrode forming method for these biosensors, as well as a method and an apparatus for quantifying using the biosensors.

#### DISCLOSURE OF THE INVENTION

According to ~~Claim 1~~ of the present invention, there is provided a biosensor for quantifying a substrate included in a sample liquid comprising: a first insulating support and a second insulating support; an electrode part comprising at

least a working electrode and a counter electrode; a specimen supply path for introducing the sample liquid to the electrode part; and a reagent layer employed for quantifying the substrate included in the sample liquid, and the electrode part, the specimen supply path, and the reagent layer exist between the first insulating support and the second insulating support, the specimen supply path is provided on the electrode part, and the reagent layer is provided on the electrode part in the specimen supply path, respectively, and the electrode part is dividedly formed by first slits provided on an electrical conductive layer which is formed on the whole or part of an internal surface of one or both of the first insulating support and the second insulating support.

Since a biosensor is constructed as described above, an electrode part can be defined easily and with a high accuracy, and variations in response of each biosensor can be reduced, resulting in a favorable response. Further, the electrode part is formed in a monolayer of electrical conductive layer, whereby troubles can be reduced and an electrode part with a smooth surface can be formed by a simple method. Since the structure of the electrode part is quite simple, it is possible to easily form biosensors having the same performance.

According to an embodiment ~~Claim 2~~ of the present invention, in the biosensor, ~~as defined in Claim 1,~~ the electrode part further comprises a detecting electrode.

Since the biosensor is constructed as described above, it is possible to make the biosensor have a better accuracy.

According to an embodiment Claim-3 of the present invention, in the biosensor, ~~as defined in Claim-2~~, the counter electrode is provided on the whole or part of the internal surface of the second insulating support, the working electrode and the detecting electrode are provided on the whole or part of the internal surface of the first insulating support, and the working electrode and the detecting electrode which are provided on the internal surface of the first insulating support are dividedly formed by the first slits provided on the electrical conductive layer.

Since the biosensor is constructed as described above, it is possible to downscale a specimen supply path, whereby a measurement can be done with a slight amount of specimen.

According to an embodiment Claim-4 of the present invention, in the biosensor, ~~as define in Claim-1 or-2~~, the electrode part is provided on the whole or part of the internal surface of only the first insulating support, and the electrode part provided on the internal surface of the first insulating support is dividedly formed by the first ~~first~~ slits provided on the electrical conductive layer.

Since the biosensor is constructed as described above, all of the electrodes are provided on the same surface, and thus the electrodes are formed only on one surface, resulting in an

easier manufacture, whereby the manufacturing costs of the biosensor can be reduced.

According to an embodiment Claim-5 of the present invention, in the biosensor ~~as defined in any of Claims 1 to 4~~, an area of the counter electrode is equal to or larger than that of the working electrode.

Since the biosensor is constructed as described above, an electron transfer reaction between the counter electrode and the working electrode is prevented to be rate-determined, thereby promoting the reaction smoothly.

According to an embodiment Claim-6 of the present invention, in the biosensor ~~as defined in any of Claims 1 to 4~~, a total of an area of the counter electrode and an area of the detecting electrode is equal to or larger than that of the working electrode.

Since the biosensor is constructed as described above, electron transfer reactions between the counter electrode as well as the detecting electrode and the working electrode are prevented to be rate-determined, thereby promoting the reactions smoothly.

According to an embodiment Claim-7 of the present invention, in the biosensor ~~as defined in Claim-6~~, the area of the detecting electrode in the specimen supply path of the biosensor is equal to the area of the counter electrode.

Since the biosensor is constructed as described above,

electron transfer reactions between the counter electrode as well as the detecting electrode and the working electrode are more reliably prevented to be rate-determined, thereby promoting the reactions smoothly.

According to an embodiment Claim 8 of the present invention, in the biosensor ~~as defined in any of Claims 1 to 7~~, a spacer is provided which has a cutout part for forming the specimen supply path and is placed on the electrode part, and the second insulating support is placed on the spacer.

Since the biosensor is constructed as described above, the position where the specimen supply path is provided is fixed, and the second insulating support is placed thereon, thereby preventing the specimen introduced to the specimen supply path from leaking from the specimen supply path.

According to an embodiment Claim 9 of the present invention, in the biosensor ~~as defined in Claim 8~~, the spacer and the second insulating support is integral.

Since the biosensor is constructed as described above, the spacer and the second insulating support is integral, thereby to enable a cost reduction and a simple manufacture.

According to an embodiment Claim 10 of the present invention, in the biosensor ~~as defined in any of Claims 1 to 9~~, an air hole leading to the specimen supply path is formed.

Since the biosensor is constructed as described above, excessive air is discharged from the air hole when the specimen

is introduced to the specimen supply path, thereby reliably introducing the specimen to the specimen supply path due to the capillary phenomenon.

According to an embodiment ~~Claim 11~~ of the present invention, in the biosensor ~~as defined in any of Claims 1 to 10~~, the reagent layer is formed by dripping a reagent, and second slits are provided around a position where the reagent is dripped.

Since the biosensor is constructed as described above, when the reagent is dripped on the electrodes for the reagent layer formation, thereby forming the reagent layer, the reagent spreads uniformly forming the reagent layer of a prescribed area at the prescribed position, whereby the reagent layer free from variations in the position and area can be formed, resulting in a correct measurement free from the variations.

According to an embodiment ~~Claim 12~~ of the present invention, in the biosensor ~~as defined in Claim 11~~, the second slits are arc shaped.

Since the biosensor is constructed as described above, the spread of the reagent is defined by the slits which have the same shapes as that of the reagent spread, thereby defining the area and the position of the reagent layer more correctly.

According to an embodiment ~~Claim 13~~ of the present invention, in the biosensor ~~as defined in any of Claims 1 to 12~~, third slits are provided for dividing the electrical conductive layer to define an area of the electrode part.

Since the biosensor is constructed as described above, when the support is initially cut at the manufacturing process of the biosensor, the area of each electrode is previously defined by the third slits, whereby the area of each electrode does not change due to the cut position of the support, thereby preventing variations in the accuracy.

According to an embodiment Claim 14 of the present invention, in the biosensor ~~as defined in Claim 13~~, shapes of the first insulating support and the second insulating support are approximately rectangular, and one third slit or two or more third slits are provided in parallel with one side of the approximate rectangle shape.

Since the biosensor is constructed as described above, the area of each electrode can be defined easily by the third slits, and the area of each electrode does not change due to deviations of the cut position when the support is cut, resulting in no variation in the accuracy.

According to an embodiment Claim 15 of the present invention, the biosensor ~~as defined in any of Claims 1 to 14~~ has information of correction data generated for each production lot of the biosensor, which correspond to characteristics concerning output of an electrical change resulting from a reaction between the reagent liquid and the reagent layer and can be discriminated by a measuring device employing the biosensor.

Since the biosensor is constructed as described above, the

measuring device can discriminate which the correction data is required, only by inserting the biosensor into the measuring device, and there is no need for a user to input the information about the correction data employing a correction chip or the like, thereby removing troubles and preventing operational errors to obtain a correct result.

According to an embodiment Claim 16 of the present invention, in the biosensor ~~as defined in Claim 15~~, one or plural fourth slits dividing the electrode part are provided, and the measuring device can discriminate the information of the correction data according to positions of the fourth slits.

Since the biosensor is constructed as described above, the measuring device can discriminate the information of the correction data by the positions of the fourth slits, the correction data can be indicated correspondingly to plural production lots, the measuring device can easily discriminate which correction data is required, by inserting the biosensor into the measuring device, whereby there is no operational trouble, resulting in preventing operational errors to obtain a correct result.

According to an embodiment Claim 17 of the present invention, in the biosensor ~~as defined in any of Claims 1 to 16~~, at least one or all of the first slits, the second slits, the third slits, and the fourth slits are formed by processing the electrical conductive layer by a laser.

Since the biosensor is constructed as described above, a high-accuracy processing is possible, the area of each electrode can be defined with a high accuracy, and further the clearance between the respective electrodes can be narrowed, resulting in a small-size biosensor.

According to an embodiment Claim 17 of the present invention, in the biosensor ~~as defined in Claim 16~~, a slit width of respective one of the first ~~first~~ slits, the second slits, the third slits, and the fourth slits is 0.005 mm to 0.3 mm.

Since the biosensor is constructed as described above, the clearance between the respective electrodes can be narrowed, resulting in a small-size biosensor.

According to an embodiment Claim 19 of the present invention, in the biosensor ~~as defined in Claims 17 or 18~~, a slit depth of respective one of the first ~~first~~ slits, the second slits, the third slits, and the fourth slits is equal to or larger than the thickness of the electrical conductive layer.

Since the biosensor is constructed as described above, there can be obtained a biosensor in which the respective electrodes are surely separated.

According to an embodiment Claim 20 of the present invention, in the biosensor ~~as defined in any of Claims 1 to 19~~, the reagent layer includes an enzyme.

Since the biosensor is constructed as described above, there can be obtained an enzyme biosensor suitable for an

inspection which employs the enzyme.

According to an embodiment ~~Claim 21~~ of the present invention, in the biosensor ~~as defined in any of Claims 1 to 19~~, the reagent layer includes an electron transfer agent.

Since the biosensor is constructed as described above, there can be obtained a biosensor suitable for an inspection utilizing a reaction of the electron transfer agent.

According to an embodiment ~~Claim 22~~ of the present invention, in the biosensor ~~as defined in any of Claims 1 to 19~~, the reagent layer includes a hydrophilic polymer.

Since the biosensor is constructed as described above, there can be obtained a high-accuracy biosensor which can easily form the reagent layer.

According to an embodiment ~~Claim 23~~ of the present invention, in the biosensor ~~as defined in any of Claims 1 to 22~~, the insulating support is made of a resin material.

Since the biosensor is constructed as described above, it is possible to manufacture a lower-cost biosensor.

According to ~~Claim 24~~ of the present invention, there is provided a thin film electrode forming method for forming a thin film electrode on a surface of an insulating support including: a roughened surface forming step of roughening the surface of the insulating support by colliding an excited gas against the surface of the insulating support in a vacuum atmosphere; and an electrical conductive layer forming step of

forming the electrical conductive layer as a thin film electrode which is composed of a conductive substance on the roughened surface of the insulating support.

Since the thin film electrode is formed as described above, a preprocessing such as a surface polishing processing is not required, whereby it is possible to form the thin film electrode by a simpler method and to form the thin film electrode with high adhesion between the support and the electrode layer.

According to an embodiment Claim-25 of the present invention, in the thin film electrode forming method ~~as defined in Claim-24~~, the roughed surface forming step comprises: a support placing step of placing the insulating support in a vacuum chamber; an evacuation step of evacuating the vacuum chamber; a gas filling step of filling up the vacuum chamber with a gas; and a colliding step of exciting the gas to be ionized and colliding the same against the insulating support.

Since the thin film electrode is formed as described above, it is possible to form the support surface suitable for forming the thin film electrode more effectively and reliably, thereby forming the thin film electrode more effectively.

According to an embodiment Claim-26 of the present invention, in the thin film electrode forming method ~~as defined in Claim-25~~, a degree of the vacuum in the evacuation step is within a range of  $1 \times 10^{-1}$  to  $3 \times 10^{-3}$  pascals.

Since the thin film electrode is formed as described above, it is possible to form the support surface suitable for forming the thin film electrode more reliably, thereby forming the thin film electrode more effectively.

According to an embodiment Claim-27 of the present invention, in the thin film electrode forming method ~~as defined in Claim-26~~, the gas is an inert gas.

Since the thin film electrode is formed as described above, the support surface can be made in a state suitable for forming the thin film electrode without denaturing the support surface.

According to an embodiment Claim-28 of the present invention, in the thin film electrode forming method ~~as defined in Claim-27~~, the inert gas is either a rare gas of argon, neon, helium, krypton, and xenon, or nitrogen.

Since the thin film electrode is formed as described above, there can be formed the thin film electrode more reliably without denaturing the support surface.

According to an embodiment Claim-29 of the present invention, in the thin film electrode forming method ~~as defined in any of Claims-24 to 28~~, the electrical conductive layer forming step comprises: a second support placing step of placing an insulating support having an already roughened surface, which has been subjected to the roughened surface forming step, in a second vacuum chamber; a second evacuation step of evacuating the second vacuum chamber; a second gas filling step of filling

up the second vacuum chamber with a second gas; and a step of exciting the second gas to be ionized and colliding the same against a conductive substance to beat out atoms of the conductive substances, to form a film on the insulating support having the already roughened surface.

Since the thin film electrode is formed as described above, a preprocessing such as a surface polishing processing is not required and the thin film electrode with higher adhesion to the support can be obtained.

According to an embodiment ~~Claim 30~~ of the present invention, in the thin film electrode forming method ~~as defined in any of Claims 24 to 28~~, the electrical conductive layer forming step comprises: a second support placing step of placing an insulating support having an already roughened surface, which has been subjected to the roughened surface forming step, in a second vacuum chamber; a second evacuation step of evacuating the second vacuum chamber; and a step of heating and evaporating a conductive substance to deposit steams as a film on the insulating support having the already roughened surface.

Since the thin film electrode is formed as described above, a preprocessing such as a surface polishing processing is not required and the thin film electrode with higher adhesion to the support can be obtained.

According to an embodiment ~~Claim 31~~ of the present invention, in the thin film electrode forming method ~~as defined in Claim 29 or 30~~,

a degree of the vacuum in the second evacuation step is within a range of  $1 \times 10^{-1}$  to  $3 \times 10^{-3}$  pascals.

Since the thin film electrode is formed as described above, there can be more reliably formed the thin film electrode with remarkably high adhesion to the support.

According to an embodiment Claim 32 of the present invention, in the thin film electrode forming method ~~as defined in any of Claims 29 to 31~~, the second gas is an inert gas.

Since the thin film electrode is formed as described above, there can be formed the thin film electrode with high adhesion to the support without denaturing the support surface and the thin film electrode itself.

According to an embodiment Claim 33 of the present invention, in the thin film electrode forming method ~~as defined in Claim 32~~, the inert gas is either a rare gas of argon, neon, helium, krypton and xenon, or nitrogen.

Since the thin film electrode is formed as described above, there can be more reliably formed the thin film electrode with high adhesion to the support without denaturing the support surface and the thin film electrode itself.

According to an embodiment Claim 34 of the present invention, in the thin film electrode forming method ~~as defined in any of Claims 29 to 33~~, the vacuum chamber and the second vacuum chamber is the same chamber.

Since the thin film electrode is formed as described above,

a facility for forming the thin film electrode can be simplified and thus the manufacturing cost of the thin film electrode can be reduced.

According to an embodiment ~~Claim 35~~ of the present invention, in the thin film electrode forming method ~~as defined in any of Claims 29 to 34~~, the conductive substance is a noble metal or carbon.

Since the thin film electrode is formed as described above, the thin film electrode is composed of not a composite material but a single substance material, thereby enabling a mass manufacture of stable electrodes, which is not influenced by the manufacturing conditions and which has a less difference in material lots.

According to an embodiment ~~claim 36~~ of the present invention, in the thin film electrode forming method ~~as defined in any of Claims 24 to 35~~, a thickness of a formed thin film electrode is within a range of 3 nm to 100 nm.

Since the thin film electrode is formed as described above, the thickness of the electrode can be thinned as much as possible, thereby to enhance a production tact as well as reduce a manufacturing cost due to a reduction of the material cost.

According to an embodiment ~~claim 37~~ of the present invention, in the biosensor ~~as defined in any of Claims 1 to 23~~, the electrical conductive layer is formed by the thin film electrode forming method ~~as defined in any of Claims 24 to 36~~.

Since the biosensor is formed as described above, the thin film electrode reflects unevenness on the support surface which is processed into a roughened surface, so that the wettability and adhesiveness between the electrode and the reagent is enhanced, resulting in a high performance biosensor.

According to an embodiment ~~Claim 38~~ of the present invention, there is provided a quantification method for quantifying, by employing the biosensor ~~as defined in any of Claims 1 to 23 and 37~~, a substrate included in a sample liquid supplied to the biosensor comprising: a first ~~first~~ application step of applying a voltage between the detecting electrode and the counter electrode or the working electrode; a reagent supplying step of supplying the sample liquid to the reagent layer; a first change detecting step of detecting an electrical change occurring between the detecting electrode and the counter electrode or the working electrode by the supply of the sample liquid to the reagent layer; a second application step of applying a voltage between the working electrode and the counter electrode as well as the detecting electrode after the electrical change is detected in the first change step; and a current measuring step of measuring a current generated between the working electrode and the counter electrode as well as the detecting electrode, to which the voltage is applied in the second application step.

Since the quantification is performed as described above, the quantification operation is started when the electrical

change occurs between the detecting electrode and the working electrode or the counter electrode of the biosensor, thereby preventing measuring errors due to the shortage of the specimen amount supplied to the reagent layer, resulting in a higher accuracy measurement. Further, when the measurable amount of specimen is supplied to the reagent layer, the measurement is performed by using the detecting electrode also as the counter electrode, thereby making the area of the electrode part smaller, and thus a quantitative analysis based on a slight amount of specimen can be performed correctly.

According to an embodiment ~~Claim 39~~ of the present invention, there is provided a quantification method for quantifying, by employing the biosensor ~~as defined in any of Claims 1 to 23 and 37~~, a substrate included in a sample liquid supplied to the biosensor comprising: a third application step of applying a voltage between the detecting electrode and the counter electrode or the working electrode as well as between the working electrode and the counter electrode; a reagent supplying step of supplying the sample liquid to the reagent layer; a first change detecting step of detecting an electrical change occurring between the detecting electrode and the counter electrode or the working electrode by the supply of the sample liquid to the reagent layer; a second change detecting step of detecting an electrical change occurring between the working electrode and the counter electrode by the supply of the sample

liquid to the reagent layer; a second application step of applying a voltage between the working electrode and the counter electrode as well as the detecting electrode after the electrical changes are detected in the first change detecting step and the second change detecting step; and a current measuring step of measuring a current generated between the working electrode and the counter electrode as well as the detecting electrode, to which the voltage is applied in the second application step.

Since the quantification is performed as described above, the quantification operation is started when the electrical change occurs between the detecting electrode and the working electrode or the counter electrode of the biosensor, thereby preventing measuring errors due to the shortage of the specimen amount supplied to the reagent layer, resulting in a higher accuracy measurement. Further, when the measurable amount of specimen is supplied to the reagent layer, the measurement is performed by using the detecting electrode also as the counter electrode, thereby making the area of the electrode part smaller, and thus quantitative analysis based on a slight amount of specimen can be performed correctly.

According to an embodiment ~~Claim 40~~ of the present invention, in the quantification method ~~as defined in Claim 38 or 39~~, the second change detecting step is followed by a no-change informing step of informing a user that no change occurs when it is detected

that no electrical change occurs between the detecting electrode and the counter electrode or the working electrode for a prescribed period of time.

Since the quantification is performed as described above, it is possible to inform a user that there is a shortage of the specimen amount supplied to the reagent layer of the biosensor, resulting in the quantification method with enhanced convenience and safety.

According to an embodiment ~~Claim 41~~ of the present invention, there is provided a quantification apparatus, to which the biosensor ~~as defined in any of Claims 1 to 23 and 37~~ is detachably connected and which quantifies a substrate included in a sample liquid supplied to the biosensor comprising: a first current/voltage conversion circuit for converting a current from the working electrode included in the biosensor into a voltage; a first A/D conversion circuit for digitally converting the voltage from the current/voltage conversion circuit; a first switch provided between the counter electrode included in the biosensor and the ground; and a control part for controlling the first ~~first~~ A/D conversion circuit and the first switch, and the control part applies a voltage between the detecting electrode and the working electrode in a state where the first switch is insulated from the counter electrode, detects an electrical change between the detecting electrode and the working electrode occurring by the sample liquid which is supplied to

the reagent layer on the specimen supply path, thereafter applies a voltage between the working electrode and the counter electrode as well as the detecting electrode in a state where the first switch is connected to the counter electrode, and measures a response current generated by applying the voltage.

Since the quantification apparatus is constructed as described above, measuring errors due to the shortage of the specimen amount supplied to the reagent layer of the specimen supply path are prevented, resulting in a higher accuracy measurement. Further, the detecting electrode of the biosensor is used also as the counter electrode at the measuring, so that the specimen supply path can be downscaled, thereby to perform a quantitative analysis of a slight amount of specimen correctly.

According to an embodiment ~~Claim 42~~ of the present invention, there is provided a quantification apparatus, to which the biosensor ~~as defined in any of Claims 1 to 23 and 37~~ is detachably connected and which quantifies a substrate included in a sample liquid supplied to the biosensor comprising: a first current/voltage conversion circuit for converting a current from the working electrode included in the biosensor into a voltage; a second current/voltage conversion circuit for converting a current from the detecting electrode included in the biosensor into a voltage; a first A/D conversion circuit for digitally converting the voltage from the first current/voltage

conversion circuit; a second A/D conversion circuit for digitally converting the voltage from the second current/voltage conversion circuit; a first selector switch for switching the connection of the detecting electrode of the biosensor to the first current/voltage conversion circuit or the ground; and a control part for controlling the first ~~first~~ A/D conversion circuit, the second A/D conversion circuit, and the first selector switch, and the control part applies a voltage between the detecting electrode and the counter electrode as well as between the working electrode and the counter electrode in a state where the first selector switch is connected to the first current/voltage conversion circuit, detects an electrical change between the detecting electrode and the working electrode as well as an electrical change between the working electrode and the counter electrode, respectively, occurring by the sample liquid which is supplied to the reagent layer provided on the specimen supply path, thereafter connects the first selector switch to the ground, applies a voltage between the working electrode and the counter electrode as well as the detecting electrode, and measures a response current generated by applying the voltage.

Since the quantification apparatus is constructed as described above, measuring errors due to the shortage of the specimen amount supplied to the reagent layer of the specimen supply path are prevented, resulting in a higher accuracy

measurement. Further, the detecting electrode of the biosensor is used also as the counter electrode at the measuring, so that the specimen supply path can be downscaled, thereby to perform a quantitative analysis of a slight amount of specimen correctly.

According to an embodiment ~~Claim 43~~ of the present invention, the quantification apparatus ~~as defined in Claim 42~~ comprises: a second selector switch for switching the connection of the working electrode of the biosensor to the second current/voltage conversion circuit or the ground, and the control part applies a voltage between the detecting electrode and the counter electrode as well as between the working electrode and the counter electrode in a state where the first selector switch is connected to the first current/voltage conversion circuit and the second selector switch is connected to the second current/voltage conversion circuit, respectively, connects the second selector switch to the ground when detecting an electrical change between the working electrode and the counter electrode, occurring by the sample liquid which is supplied to the reagent layer provided on the specimen supply path, and when thereafter detecting an electrical change between the detecting electrode and the working electrode, in a state where the second selector switch is connected to the second current/voltage conversion circuit and the first selector switch is connected to the ground, applies a voltage

between the working electrode and the counter electrode as well as the detecting electrode, and measures a response current generated by applying the voltage.

Since the quantification apparatus is constructed as described above, measuring errors due to the shortage of the specimen amount supplied to the reagent layer of the specimen supply path are prevented, resulting in a higher accuracy measurement. Further, the detecting electrode of the biosensor is used also as the counter electrode at the measuring, so that the specimen supply path can be downscaled, thereby to perform a quantitative analysis of a slight amount of specimen correctly.

According to an embodiment Claim 44 of the present invention, the quantification apparatus ~~as defined in Claim 42 or 43~~ comprising an informing means for informing a user that no change occurs, when the sample liquid is supplied to the reagent layer of the specimen supply path, and the control part detects that an electrical change occurs between the working electrode and the counter electrode but no electrical change occurs between the detecting electrode and the working electrode or the counter electrode.

Since the quantification apparatus is constructed as described above, it is possible to inform a user of the shortage of the specimen amount supplied to the reagent layer of the specimen supply path of the biosensor, resulting in the

blood glucose concentration of 40-600 mg/dl are compared.

Figures 21(a)-(b) are exploded perspective views of a conventional biosensor.

Figure 22 is a diagram illustrating a state where a biosensor is inserted in a measuring device.

Figure 23 is a top view illustrating a state where slits are formed in the electrical conductive layer which is provided on a sensor wafer according to the third embodiment.

Figures 24(a)-(c) are top views illustrating states of electrodes of a biosensor in a manufacturing method according to the third embodiment.

Figure 25 is a diagram illustrating the concept of a cross-sectional structure of a conventional biosensor.

#### BEST MODE TO EXECUTE THE INVENTION

Hereinafter, embodiments of the present invention will be described with reference to the figures. The embodiments which are described here are merely examples, and the present invention is not necessarily restricted thereto.

#### (Embodiment 1)

A biosensor A as defined in ~~Claims 1 to 10~~ of the present invention will be described as a first embodiment with reference to the figures.

Figures 1(a) to 1(c) are exploded perspective views of the biosensor A according to the first embodiment of the present

conductive material such as noble metal for example gold or palladium and carbon as the material, it take no trouble of successively printing and laminating a silver paste, a carbon paste and the like on the support 1 as in the prior art, whereby it is possible to form the electrode part with a smooth surface by a simple method. Further, since the slits 4a and 4b are formed on the conductive layer 2 which is provided on the support 1 by the laser, it is possible to define the area of each electrode with a higher accuracy. The clearance between the respective electrodes can be considerably reduced to downsize the specimen supply path, thereby enabling the measurement based on a trace quantity of specimen while this could not be measured conventionally. Further, since the structures of the electrodes are very simple, a biosensor having the same performance can be easily formed.

(Embodiment 2)

A biosensor B according to ~~claims 11 and 12~~ of the present invention will be described as a second embodiment.

Figures 3(a)-(c) are perspective views illustrating the biosensor B in the order of the manufacturing process, and figure 4 is a diagram illustrating a specimen supply path of the biosensor B.

First, the structure of the biosensor B will be described.

Numeral 21 denotes an insulating support which is composed of polyethylene terephthalate or the like. Numeral 22 denotes an electrical conductive layer which is formed on the whole

individual biosensor.

However, the so-formed biosensor X has a problem in that when the plural biosensors are to be cut into individual biosensors, there are some cases where the cutting cannot be performed on the cutting plane lines, resulting in deviations from the cutting plane lines 3110. This will be described in more detail. Figure 24(a) is a diagram illustrating states of the electrodes in a case where the cutting is correctly performed. Figure 24(b) is a diagram illustrating states of the electrodes when the cutting position is deviated toward left from the cutting plane line 3110. Figure 24(c) is a diagram illustrating states of the electrodes when the cutting position is deviated toward right from the cutting plane line 3110. Since the areas of the working electrode 3105 and the counter electrode 3106 are decided by the cutting position of the individual wafer Q, changes in the areas of the working electrode 3105 and the counter electrode 3106 occur when the cutting position is deviated from the cutting plane line 3110 as shown in the figures, resulting in variations in resistance values of the respective electrodes. Therefore, values of currents flowing the electrodes change, whereby the accuracy of the biosensor X get worse.

Here, a biosensor C according to ~~Claims 13 and 14~~ of the present invention, which has for its object to solve this problem will be described as a third embodiment.

(Embodiment 4)

A biosensor D according to ~~Claims 15 and 16 of~~ the present invention will be described as a fourth embodiment.

Figures 9(a)-(c) are perspective views illustrating the biosensor D in the order of a manufacturing process. Figures 10(a)-(h) are top views exemplifying the formation of fourth slits of the biosensor D. Figure 22 is a diagram illustrating a state where the biosensor D is inserted into a measuring device.

First, components of the biosensor D will be described.

Numeral 61 denotes an insulating support composed of polyethylene terephthalate or the like. Numeral 62 denotes an electrical conductive layer which is formed on the whole surface of the support 61 and is composed of an electrical conductive material such as a noble metal, for example gold or palladium, and carbon. Numerals 63a, 63b, 63c and 63d denote first slits provided in the electrical conductive layer 62. Numerals 65, 66, and 67 denote electrodes which are formed by dividing the electrical conductive layer 62 by the first slits 63a, 63b, 63c and 63d, i.e., a working electrode, a counter electrode, and a detecting electrode as an electrode for confirming whether the specimen is surely drawn into a specimen supply path, respectively. Numerals 64a, 64b, and 64c denote fourth slits which divide the counter electrode 66, the detecting electrode 67, and the working electrode 65, respectively. Numeral 68 denotes a spacer which covers the

preferable that each slit provided on the electrical conductive layer is processed by the laser, the width of each slit is 0.005 mm - 0.3 mm, and the depth of each slit is equal to or larger than the thickness of the electrical conductive layer, ~~as defined in claims 16 to 18 of the present invention.~~

Further, it is preferred that the reagent layer provided in any of the biosensors A, B, C, and D should include enzyme, an electron transfer agent, or a hydrophilic polymer, ~~as defined in Claims 19 to 21 of the present invention.~~

In addition, it is preferable that the insulating support employed in any of the biosensors A, B, C, and D is made of a resin material, ~~as defined in Claim 22 of the present invention.~~

(Embodiment 5)

A thin film electrode forming method ~~as defined in Claims 23 to 35~~ of the present invention will be described as a fifth embodiment with reference to the figures. When the thin film electrode method described in the fifth embodiment is applied when the electrode parts of any of the biosensors A, B, C, and D according to the above-described first to fourth embodiments are formed, a biosensor ~~as defined in Claim 36~~ of the present invention can be obtained.

Figure 11 is a schematic diagram showing a state of a biosensor, where a thin film electrode is formed by implementing the thin film electrode forming method according to this embodiment and a reaction reagent layer are laid out

substrate ~~as defined in Claim 38 of the present invention~~ and a quantification apparatus for quantifying a substrate ~~as defined in Claim 41 of the present invention~~, which employ any of the biosensors A, B, C, and D, for which the electrical conductive layers are formed by employing the above-described thin film electrode forming method according to the fifth embodiment will be described. while the biosensor A as described in the first embodiment is used as a biosensor employed in a following description, the biosensor to be used is not restricted thereto.

Figure 13 is a diagram illustrating structures of the biosensor and the quantification apparatus which is employed in the quantification method employing the biosensor. In the figure, the same reference numerals as those shown in figure 1 denote the same or corresponding parts.

It is a system in which the biosensor A is used in a state where it is connected to a quantification apparatus M1, and the quantification apparatus M1 measures the amount of an included substrate from a specimen supplied to the biosensor A.

In the quantification apparatus M1, numerals 115a, 115b, and 115c denote connectors connected to a working electrode 5, a detecting electrode 7, a counter electrode 6 of the biosensor A, respectively, numeral 116a denotes a switch provided between the connector 115c and the ground (which means a constant potential electrodeposition and can be not always "0". The same goes for in the present specification.), numeral 118a

electrode 7 is at least equivalent to the area of the working electrode 5, the electron transfer reaction between the electrodes is prevented from being rate-determined, thereby to promote the reaction smoothly. At the same time, the capacity of the specimen supply path can be downsized, whereby the quantitative analysis based on a slight amount of specimen, which was conventionally impossible, can be performed properly. Further, when the area of the detecting electrode 7 and that of the counter electrode 6 are equivalent, the electron transfer reaction between the electrodes is performed uniformly, thereby obtaining a more satisfactory response.

(Embodiment 7)

Hereinafter, a quantification method for quantifying a substrate ~~as defined in Claim 40 of the present invention~~ and a quantification apparatus for quantifying a substrate ~~as defined in Claims 42 to 44 of the present invention~~, which employ any of the biosensors A to D whose electrical conductive layers are formed by employing the thin film electrode forming method described in the fifth embodiment but which are different from those of the above-described sixth embodiment will be described. A biosensor which is employed in a following description is supposed to be the biosensor A described in the first embodiment.

Figure 16 is a diagram illustrating structures of the biosensor A and a quantification apparatus employed in the

the counter electrode 6 and the working electrode 5 by the supply of the specimen to the specimen supply path but no current is thereafter generated between the counter electrode 6 and the detecting electrode 7 for the prescribed period of time, the CPU 120 judges that there is a shortage of the specimen amount, and this is displayed on the LCD 121. Even when the specimen is supplemented to the specimen supply path after the LCD 121 once displays that there is a shortage of the specimen supply, the CPU 120 does not start the quantification operation.

As described above, according to the quantification method employing the biosensor in the seventh embodiment of the present invention, when the specimen is drawn into the specimen supply path of the biosensor A, and electrical changes occur between the counter electrode 6 and the working electrode 5 while no electrical change occurs between the counter electrode 6 and the detecting electrode 7, the quantification apparatus M3 displays on the LCD 121 that there is a shortage of the specimen supply and informs a user of the fact, thereby enhancing the convenience and safety at the measuring.

(Embodiment 8)

Hereinafter, a quantification method for quantifying a substrate ~~as defined in Claim 39 or 40 of the present invention~~ and a quantification apparatus for quantifying a substrate ~~as defined in Claims 42 to 44 of the present invention~~, which employ any of the biosensors A to D whose electrical conductive